REMARKS

Claims 1-14 are currently pending in the application. In order to advance prosecution, Applicants have canceled claims 1 and 14, amended claims 2-13, and added claim 15-20. A clean version of the amended claims is shown above. A version of the amended claims with markings to show the changes made appears as Appendix A.

The amendments to the pending claims were made to clarify the scope of coverage and more particularly point out and distinctly claim the present invention. These amendments are made without prejudice, do not constitute amendments to overcome any prior art rejections under U.S.C. § 102, and do not present any new matter. For example, the phase "average optical density" appears, *inter alia*, at page 8 of the specification, where a non-limiting calculation of the average optical density is described as "dividing the total optical density of the stained target protein by the total number of pixels of the ethyl green or methylene blue stained sample." Additional descriptions of "average optical density" can be found throughout the specification.

Cancellation of claims 1 and 14 makes no admission regarding the patentability of this subject matter and should not be so construed. Applicant reserves the right to pursue this subject matter in this or in any other appropriate patent application.

Replacement Figures 2, 3, and 6 have been amended to comply with C.F.R. § 1.84. Specifically, the lines, numbers, and reference characters have been changed so that they comply with the rules and the comments on the Draftsperson's report. No new matter has been introduced by any of these amendments to the Figures.

Additionally, attached herewith as Appendix C are copies of 3 references that the Examiner believes were not included with Applicant's Information Disclosure Statement.

Applicant requests that the Examiner consider the entirety of each document and make them of record in this application.

Discussion of the 35 U.S.C. § 112, ¶ 2 Rejection

Claims 1-14 are rejected under 35 U.S.C. § 112, second paragraph, for being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The rejection is respectfully traversed.

With respect to claim 1, subpart b), the Office Action stated that the phrase "the optical density" has no antecedent basis. In addition, in the same claim, subpart d), lines 2-3, the Office Action stated that the second occurrence of the phase "the target protein" lacks clear antecedent support. With respect to claim 5, the Office Action stated that the term "consistent" is indefinite. With respect to claim 7 and 14, the Office Action stated that the phrase "ELISA" is indefinite. With respect to claim 8-10, the Office action stated that the phrase "the target protein is normalized to the amount of protein in the cell pellet" lacks clear antecedent support. With respect to claim 11, the Office Action stated that the phrase "optical density of the staining in the cells" is vague and indefinite. With respect to claim 12, the Office Action stated that the phrase "the biological sample" lacks clear antecedent basis. In addition, in the same claim, the Office Action stated that the phrase "a multiplicity of stains used to stain the cells" lacks antecedent support. Applicant has amended the above-referenced claims to better clarify the claimed invention, thereby overcoming the Examiner's rejections.

In addition, with respect to claim 3, the Office Action stated that the recitation of the terms "EGFR," "AKT," and "MAP" are indefinite because acronyms or abbreviations must be recited at least one time in a set of claims. With respect to "EGFR" and "MAP," applicant has

amended the claims, thereby overcoming the Examiner's rejection. With respect to "AKT," however, applicant traverses the rejection. Applicant respectfully points out that "AKT" is not an abbreviation, but rather the non-abbreviated name of an oncogenic protein. *See, e.g.*, *Specification* at 2, 4-5, 9, and 13. Therefore, the term is not indefinite as recited in the claim. In view of the above, Applicant respectfully requests withdrawal of the 35 U.S.C. § 112, ¶ 2 rejection.

Discussion of the 35 U.S.C. § 102 Rejection

Claims 1-11 and 13-14 stand rejected under 35 U.S.C. § 102(b) for being anticipated by U.S. Patent No. 5,846,749 (the "'749 patent"). In addition, claims 1-3, 6, and 11-13 stand rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,007,996 (the "'996 patent"). Applicants respectfully traverse these rejections with the following arguments.

Under 35 U.S.C § 102, a claim is anticipated only if each and every element as set forth in the claim is found in a single art reference. M.P.E.P. § 2131; *Verdegall Bros. V. Union Oil Co.*, 814 F.2d 628, 631, 2 U.S.P.Q.2d (BNA) 1051, 1053 (Fed. Cir. 1987). The identical invention must be shown in as complete detail as is contained in the claim. M.P.E.P. § 2131; *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 U.S.P.Q.2d 1913, 1920 (Fed. Cir. 1989).

The instantly claimed invention is directed towards methods for determining the quantity of a target protein in a biological sample. This method requires, among other things, that a calibration curve be generated relating the quantity of the target protein with an average optical density of target protein-specific staining in the sample. The calibration curve also needs to be generated with at least two control cell pellets that have different quantities of the target protein.

The '749 patent does not anticipate the present invention because it does not teach or suggest every element as set forth in the claims. Specifically, it does not teach a method of determining an "average optical density of target protein-specific staining." Instead, the '749 patent discloses, *inter alia*, a method of quantitating a surface membrane or cytosolic protein wherein fixed cells are stained with a label specific to a target protein that can be quantitated by image analysis, the signal value for a sample is determined by means of image analysis, and the signal value is compared with control values obtained from cells with known amounts of target protein. *See '749 patent*, column 16, lines 6-25. The '749 patent also details a method for determining the amount of protein in the control cells by quantitative Western blot analysis, wherein stained bands in lanes corresponding to either a specific number of control cells (specifically 10^6cells) or to purified target protein standards are quantitated and used for generating a calibration curve relating staining intensity to target protein concentration. *See, e.g., id.* at column 11, lines 27-67. The methods of the '749 patent, however, do not identify the average optical density of target protein-specific staining as required by the instant claims.

Image analysis according to the '749 patent is accomplished by analyzing the immunostaining of the entire microscopic field, defined as an area of approximately 500 square microns. *Id.* at column 6, lines 3-5. Tissue in this microscopic field will include the cells of interest, as well as additional cells and extracellular material. This constitution of the field was even noted in the '749 patent, where it was observed that "stromal cells, connective tissue, lymphocytes, and normal tissue were not immunostained" in sample preparations, regardless of whether the sample was prepared from frozen tissue or paraffin-embedded material. *Id.* at column 13, lines 21-24. In order to compensate for this problem, the '749 patent discloses the analysis of 5 different fields, and suggests that usually 2-10 different fields be analyzed with the

results averaged together. *Id.* at column 5, line 67-column 6, line 3 and column 13, line 51-54. This practice is presumably used to compensate for variability in staining observed when only one microscopic field is analyzed. In contrast, to determine the "average optical density of target protein-specific staining," as required by the instant invention, it is necessary that any extraneous material in the field *not* be included in the calculation. Therefore, because the measurement of entire microscopic field is taught be the'749 patent, it cannot anticipate the instant claims.

In addition, the difficulty in accurately quantitating the amount of target protein using the methods of the '749 patent is exacerbated by differential staining based on sample preparation. For example, the inventors of the '749 patent state that immunohistochemical samples consisting of tumor cells in paraffin sections were not as homogenously stained as those samples prepared from cells frozen in OCT cryosection media. *Id.* at column 13, lines 14-16. Even though the tissue samples were formalin-fixed and embedded in paraffin, the control cells (*i.e.*, the cells whose target protein content was quantified) were frozen in OCT cryosection media in preparation for immunostaining because of the relative homogeneity of the staining of these samples. *Id.* at column 10, lines 39-46. In order to compensate for the difference in staining, the '749 patent describes comparison of formalin-fixed paraffin-embedded cells with cells prepared as frozen samples from the same culture, and determines that the apparent HER-2/*neu* content of the paraffin-embedded cells, based on immunostaining, was 61.3% of that observed in the frozen cells. *Id.* at column 14, lines 1-6. This corrective factor was then used to calculate the quantity of target protein in biological tissue samples, "*assuming* that the reduction in immunostaining intensity of breast cancer specimens [would be] close to that observed in similarly treated culture cells," without identifying whether cells grown in culture would stain any differently than cells in a tissue sample. *Id.* at column 14, lines 6-25 (emphasis added). Thus, the '749 patent teaches

a guess based on the inventors' stated assumption, and does not teach an accurate system for quantitating target proteins in a biological sample, let alone the determination of the "average optical density of target protein-specific staining" required by the instant claims.

The inability of the '749 patent to accurately and reliably quantitate target protein amounts was recognized in the art prior to the date of filing of the instant application. International application WO 00/23799 ("Smith"), which was filed by Steven J. Smith and was included in the present application's Information Disclosure Statement, discussed the deficiencies of the '749 patent.[1] Smith relates that the '749 patent was an attempt to improve the accuracy of the measurement of proteins in cancer tissue. *Smith* at 2, lines 29-32. However, as discussed above, Smith observed the limitations of the '749 patent, in that the "fixation conditions of the reference cells and the tissue were different, there were no immunostained paraffin sections for the reference cells, and the frozen tissue stained more intensely than the paraffinized tissue" using the '749 patent methods. *Id.* at lines 16-20. Smith avers that those skilled in the art did not believe that the disclosure of the '749 patent taught an accurate method of quantitating target protein. *Id.* Thus, Smith shows that the skilled artisan would recognize that the '749 patent does not teach and cannot anticipate the instantly claimed invention.

Turning to the '996 patent, this patent also does not anticipate the instant invention, because it does not teach, *inter alia*, the generation of a calibration curve. The '996 discloses, among other things, a method of *in situ* analysis of a biological sample comprising the steps of

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[1] The Smith application actually discusses a Cancer Research paper written by Press *et al.* (Press, *et al.*, 1993, *Cancer Res.* 53:4960-70 ("Press")), but a comparison of Press and the '749 patent reveals that the disclosure of the '749 patent contains the data, and only the data, from Press. For example, Tables 1, 2 and 3 in Press correspond to Tables 2, 1, and 3 in the '749 patent, respectively. In fact, even though the '749 patent contains no figures, there are references to figures in the specification, the description of which correlate to the figures in Press (*see, e.g.*, Legend (a) in Table 1 of the '749 patent). In consideration of the similarity in subject matter, Smith's critique of the Press reference is equally applicable to the '749 patent. The Smith and Press references are attached as Appendix B.

staining the biological sample with at least three stains, and collecting spectral data from the stained biological sample, where the spectral data collection device can collect data from all the stains. *See '996 patent* at column 55, line 66-column 56, line 24. The methods of the '996 patent do not involve generating any calibration curve, let alone a calibration curve that relates the quantity of the target protein with an optical density of the target protein-specific staining. As acknowledged by the Office Action on page 7, the '996 patent does disclose the use of an external calibration "to account for day-to-day variations experienced when staining is attempted." *Id.* at column 38, lines 4-7. However, even though the calibration material can be control cells stained at the same time as the sample, the calibration material can, alternatively, be an optical density reference material. *See id.* at column 38, lines 20-24. Such an optical density reference material would have no utility in calibrating quantitation of target protein amount. Instead, the teaching of the '996 patent in this regard demonstrates that the calibration material is used to calibrate the spectral data collection device, and not to generate a calibration curve to relate the quantity and average optical density of a stained target protein. Thus, the '996 patent teaches neither a calibration curve nor any calibration method for relating the amount of a target protein in a sample to a standard amount of a specifically-stained target protein. For at least these reasons, the '996 patent does not anticipate the claimed invention.

Applicants respectfully contend that rejection on this 35 U.S.C. § 102 ground has been traversed by their argument herein, and request that this rejection be withdrawn.

Conclusion

In view of the above remarks, the application is considered to be in good and proper form for allowance and the Examiner is respectfully requested to pass this application to issue. If, in

the opinion of the Examiner, a telephone call would expedite the prosecution of this application, the Examiner is invited to call the undersigned attorney.

Respectfully Submitted,

Date: September 3, 2002

Andrew W. Williams, Ph.D.
Reg. No. 48,644

APPENDIX A: REWRITTEN CLAIM WITH MARKINGS TO SHOW CHANGES

2. [Amended] The method of claim [1] 15, wherein the target protein is a protein that is [specifically] expressed in malignant cells in an animal.

3. [Amended] The method of claim 2, wherein the target protein is Her-2/*neu*, Her-3, Her-4, estrogen receptor, prostate-specific antigen, Epidermal Growth Factor Receptor ("EGFR"), AKT, p13 kinase [and] or Mitogen-Activated Protein Kinase ("MAP kinase").

4. [Amended] The method of claim [1] 15, wherein the plurality of control cell pellets are prepared from cultured cell lines.

5. [Amended] The method of claim 4, wherein the cultured cell line expresses a reproducible [consistent] amount of the target protein.

6. [Amended] The method of claim 15 [1], wherein the quantity of said [amount of] target protein from each of the control [in the] cell pellets is determined using an immunological reagent [immunohistochemically].

7. [Amended] The method of claim 6, wherein the quantity of said [amount of] target protein from each of the control cell pellets is determined by Enzyme Linked Immunosorbent Assay ("ELISA") [assay].

8. [Amended] The method of claim 15 [1], wherein the quantity of said [amount of] target protein from each of the control cell pellets is normalized to the total amount of protein in the cell pellet.

9. [Amended] The method of claim 8, wherein the quantity of said [amount of] target protein from each of the control cell pellets is normalized to the total amount of protein per cell.

McDonnell Boehnen Hulbert & Berghoff
300 South Wacker Drive, 32nd Floor
Chicago, IL 60606
(312) 913-0001

10. [Amended] The method of claim 8, wherein the quantity of said [amount of] target protein in the calibration curve is expressed as number of target protein molecules per cell.

11. [Amended] The method of claim 15 [1], wherein the average optical density of the target protein-specific staining [staining in the cells of the biological sample] is determined using image analysis.

12. [Amended] The method of claim 11, wherein said biological sample is stained with a multiplicity of stains, and wherein the image analysis is performed by splitting a signal comprising an [the] optical density of the stained target protein in said biological sample into a multiplicity of signals that are processed using optical filters having different absorption and transmittance properties, so that each signal is specific for one of said [a] multiplicity of stains used to stain the cells in the biological sample.

13. [Amended] The method of claim 15 [1], wherein the detectable label is a chromogen [chromagen] or a fluorophore.

15. A method for determining the quantity of a target protein in a biological sample comprising a cell, the method comprising the steps of:

(a) determining the quantity of said target protein in a plurality of control cell pellets, wherein said quantity is determined in a first portion from each of the control cell pellets, and wherein the quantity of the target protein from each of the control cell pellets is not the same;

(b) immunohistochemically staining said target protein in a second portion from each of the control cell pellets using a detectably labeled antibody directed against said target protein;

(c) determining an average optical density of target protein-specific staining in the second portion from each of the control cell pellets;

(d) generating a calibration curve relating said quantity of said target protein as determined in (a) with said average optical density of target protein-specific staining as determined in (c) for the plurality of control cell pellets;

(e) immunohistochemically staining said target protein from a portion of cells from said biological sample using said detectably labeled antibody directed against said target protein;

(f) determining an average optical density of target protein-specific staining in said portion of cells from said biological sample;

(g) determining the quantity of said target protein in said biological sample by comparing the average optical density of target protein-specific staining as determined in step (f) in said portion of cells from said biological sample to the calibration curve as generated in step (d), wherein the quantity of said target protein is derived from the calibration curve.

16. The method of claim 15, wherein said biological sample is a tissue or cell sample removed from a subject.

17. The method of claim 15, wherein the plurality of control cell pellets are not embedded in paraffin.

18. The method of claim 15, wherein the plurality of control cell pellets are not immobilized in a hydrophilic matrix.

19. The method of claim 15, wherein the calibration curve in linear.

20. The method of claim 15, wherein the immunohistochemically staining of (e) is performed with the same reagents as is used for the immunohistochemically staining of (b).

Form PTO 948 (Rev. 03/01) U.S. DEPARTMENT OF COMMERCE - Patent and Trademark Office Application No. 9/760,120

NOTICE OF DRAFTSPERSON'S PATENT DRAWING REVIEW

The drawing(s) filed (insert date) 1-12-01 are:

A. ☐ approved by the Draftsperson under 37 CFR 1.84 or 1.152.

B. ☑ objected to by the Draftsperson under 37 CFR 1.84 or 1.152 for the reasons indicated below. The Examiner will require submission of new, corrected drawings when necessary. Corrected drawing must be sumitted according to the instructions on the back of this notice.

1. DRAWINGS. 37 CFR 1.84(a): Acceptable categories of drawings: Black ink. Color.
   - ___ Color drawings are not acceptable until petiton is granted. Fig(s) _____
   - ___ Pencil and non black ink not permitted. Fig(s) _____
2. PHOTOGRAPHS. 37 CFR 1.84(b)
   - ___ 1 full-tone set is required. Fig(s) _____
   - ___ Photographs may not be mounted. 37 CFR 1.84(e)
   - ___ Poor quality (half-tone). Fig(s) _____
3. TYPE OF PAPER. 37 CFR 1.84(e)
   - ___ Paper not flexible, strong, white, and durable. Fig(s) _____
   - ___ Erasures, alterations, overwritings, interlineations, folds, copy machine marks not accepted. Fig(s) _____
   - ___ Mylar, velum paper is not acceptable (too thin). Fig(s) _____
4. SIZE OF PAPER. 37 CFR 1.84(f): Acceptable sizes:
   - ___ 21.0 cm by 29.7 cm (DIN size A4)
   - ___ 21.6 cm by 27.9 cm (8 1/2 x 11 inches)
   - ___ All drawing sheets not the same size. Sheet(s) _____
   - ___ Drawings sheets not an acceptable size. Fig(s) _____
5. MARGINS. 37 CFR 1.84(g): Acceptable margins:

   Top 2.5 cm Left 2.5cm Right 1.5 cm Bottom 1.0 cm SIZE: A4 Size

   Top 2.5 cm Left 2.5 cm Right 1.5 cm Bottom 1.0 cm SIZE: 8 1/2 x 11

   Margins not acceptable. Fig(s) _____

   _____ Top (T) _____ Left (L)

   _____ Right (R) _____ Bottom (B)
6. VIEWS. 37 CFR 1.84(h)

   REMINDER: Specification may require revision to correspond to drawing changes.

   Partial views. 37 CFR 1.84(h)(2)
   - ___ Brackets needed to show figure as one entity. Fig(s) _____
   - ___ Views not labeled separately or properly. Fig(s) _____
   - ___ Enlarged view not labeled separetely or properly. Fig(s) _____
7. SECTIONAL VIEWS. 37 CFR 1.84 (h)(3)
   - ___ Hatching not indicated for sectional portions of an object. Fig(s) _____
   - ___ Sectional designation should be noted with Arabic or Roman numbers. Fig(s) _____
8. ARRANGEMENT OF VIEWS. 37 CFR 1.84(i)
   - ___ Words do not appear on a horizontal, left-to-right fashion when page is either upright or turned so that the top becomes the right side, except for graphs. Fig(s) _____
9. SCALE. 37 CFR 1.84(k)
   - ___ Scale not large enough to show mechanism without crowding when drawing is reduced in size to two-thirds in reproduction. Fig(s) _____
10. CHARACTER OF LINES, NUMBERS, & LETTERS. 37 CFR 1.84(i)
    - ___/ Lines, numbers & letters not uniformly thick and well defined, clean, durable, and black (poor line quality). Fig(s) 2 3
11. SHADING. 37 CFR 1.84(m)
    - ___ Solid black areas pale. Fig(s) _____
    - ___ Solid black shading not permitted. Fig(s) _____
    - ___ Shade lines, pale, rough and blurred. Fig(s) _____
12. NUMBERS, LETTERS, & REFERENCE CHARACTERS. 37 CFR 1.84(p)
    - ___/ Numbers and reference characters not plain and legible. Fig(s) 2 3 6
    - ___ Figure legends are poor. Fig(s) _____
    - ___ Numbers and reference characters not oriented in the same direction as the view. 37 CFR 1.84(p)(1) Fig(s) _____
    - ___ English alphabet not used. 37 CFR 1.84(p)(2) Figs _____
    - ___ Numbers, letters and reference characters must be at least .32 cm (1/8 inch) in height. 37 CFR 1.84(p)(3) Fig(s) _____
13. LEAD LINES. 37 CFR 1.84(q)
    - ___ Lead lines cross each other. Fig(s) _____
    - ___ Lead lines missing. Fig(s) _____
14. NUMBERING OF SHEETS OF DRAWINGS. 37 CFR 1.84(t)
    - ___ Sheets not numbered consecutively, and in Arabic numerals beginning with number 1. Sheet(s) _____
15. NUMBERING OF VIEWS. 37 CFR 1.84(u)
    - ___ Views not numbered consecutively, and in Arabic numerals, beginning with number 1. Fig(s) _____
16. CORRECTIONS. 37 CFR 1.84(w)
    - ___ Corrections not made from prior PTO-948 dated _____
17. DESIGN DRAWINGS. 37 CFR 1.152
    - ___ Surface shading shown not appropriate. Fig(s) _____
    - ___ Solid black shading not used for color contrast. Fig(s) _____

COMMENTS

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ATTACHMENT TO PAPER NO. _____